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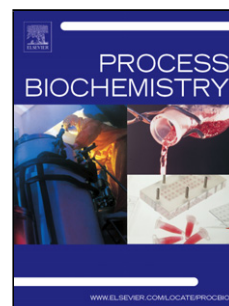
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**Sustainable microbial biosurfactants and bioemulsifiers for commercial exploitation**

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## Highlights

- Emulsifiers and surfactants aid in the formation and stabilization of emulsions
- Emulsion-based formulations are exploited in both food and cosmetic industries
- Biosurfactants/bioemulsifiers have potential for replacing conventional surfactants

## Abstract

Emulsion-based formulations are widely exploited in medicine, food and cosmetics, where surfactants and emulsifiers play contrasting, but equally important roles. Both are able to adsorb at the oil and water interface enabling dispersion of droplets of one phase in the second. The main function of surfactants is lowering the interfacial tension, while emulsifiers, adsorb more slowly to the droplet surface and give longer-term stability. High toxicity and high ecological impact of many synthetic surfactants/emulsifiers have led to interest in other, natural molecules such as biosurfactants and bioemulsifiers. These bio-based surface-active agents are interesting for the main industries linked to human health – pharmaceuticals, food and cosmetics, with several having been discovered and already widely applied. However, the commercialisation of the production of bio-surfactants/emulsifiers is often limited by optimisation difficulties, high purification costs and low yields, all of which have an impact on their wider application. Therefore, much effort is required to find stable and cost effective methods to produce bio-surfactants/emulsifiers on a large scale. This review focuses on the main properties of bio-emulsifiers and bio-surfactants, followed by an overview of their current use in consumer product formulations. Finally, the potential of bio-surfactants/emulsifiers to replace the currently used counterparts will be assessed.

Keywords: emulsion, bioemulsifiers, biosurfactants, natural emulsifiers, emulsions in food, emulsions in cosmetics

## Abbreviations:

ACETEM – acetic acid esters of monoglycerides  
 CITREM – citric acid esters of monoglycerides  
 CLA – conjugated linoleic acid  
 CMC – critical micelle concentration  
 CSL – calcium stearoyl lactylate  
 DATEM – diacetyl tartaric acid esters of monoglycerides  
 DMPC – 1,2-dimyristoylphosphatidylcholine;  
 DOPC – 1,2-dioleoylphosphatidylcholine;  
 DOPE – 1,2-dioleoyl-sn-glycero- phosphoetamine,  
 DPPG – 1,2-dipalmitoylphosphatidylcholine;  
 DSPC – 1,2-distearoylphosphatidylcholine;  
 DSPG – 1,2-distearoylphosphatidylglycerol  
 FA – fatty acids  
 FDA – United States Food and Drug Administration  
 GNP – gold nanoparticles  
 HA – hyaluronic acid  
 HLB – hydrophile-lipophile balance  
 IFT – interfacial tension  
 INCI – International Nomenclature of Cosmetic Ingredients  
 LAB – lactic acid bacteria  
 LACTEM – lactic acid esters of monoglycerides  
 LMW – low molecular weight  
 LPs – lipopeptides  
 MELs – mannosylerythritol lipids  
 MG – monoglycerides  
 O/W – oil-in-water emulsion  
 O/W/O – oil-in-water-in-oil emulsion  
 PAH – polycyclic aromatic hydrocarbons  
 PEG – poly(ethylene glycol)  
 PG – phosphatidylglycerol;  
 PGE – polyglycerol esters of fatty acids  
 PGMS – propylene glycol esters of fatty acids  
 PKC – protein kinase C  
 RLs – Rhamnolipids  
 rPA – recombinant protective antigen  
 SLs – sophorolipids  
 SMG – succinic acid esters of monoglycerides  
 SMS – sorbitan monostearate  
 SSL – sodium stearoyl lactylate  
 STS – sorbitan tristearate  
 TLs – threhalolipids  
 W/O – water-in-oil emulsion  
 W/O/W – water-in-oil-in-water emulsion  
 WPI – whey protein isolate  
 XG – Xanthan gum

## 1. Introduction

In recent years, increasing emphasis in the food and cosmetic industry on green ingredients has been observed. Demands on environmental resources as well as growing consumer awareness on using natural ingredients over synthetic additives (“clean-label” products) have led to extensive studies on screening biological sources (such as plants, agri-food waste, microorganisms) for surfactants and emulsifiers of a natural origin.

Often emulsifiers/surfactants derived from plants are referred to as natural whilst those obtained from microorganisms (bacteria, yeast) are termed bio-emulsifiers/bio-surfactants, however, it is not uncommon to see these terms used interchangeably. This review will focus on the common surfactants and emulsifiers that are used in pharmaceutical, cosmetic and food formulations. Bio-surfactants and bio-emulsifiers that could be substituted for currently used surfactants and emulsifiers will be discussed, emphasizing the similarities and differences between them.

Emulsifiers and surfactants are surface-active molecules that adsorb to oil-water (emulsion) and air-water (foam) interfaces and aid in the formation and stabilization of emulsions and foams. The two types of molecule can be distinguished based on their structure and physico-chemical properties. Emulsifiers refer to biopolymers, usually proteins or polysaccharides. They are able to reduce surface tension to form emulsion droplets and foam bubbles and to form a steric-stabilizing adsorbed layer at the interface. Surfactants, on the other hand are low molecular weight molecules that are amphiphilic in nature, with a hydrophilic head group and a hydrophobic tail. Their surface activity derives from a balance between hydrophobic and hydrophilic moieties in the molecule. Being low molecular weight, surfactants adsorb rapidly at newly formed interfaces and stabilize oil droplets and air bubbles when they are small, and therefore inherently more stable. To the contrary, this must be balanced against the reduced steric stabilizing ability of the thinner surfactant adsorbed layer compared to that of polymeric

emulsifiers. In consumer product formulations, emulsifiers and surfactants are used in combination to exploit the desirable properties of the two.

Emulsion-based formulations are widely exploited in both food and cosmetic industries. The essential role of surface-active components in these formulations has led to a high interest in identifying bio-surfactants and bio-emulsifiers for these industries. This trend is driven by a consumer desire for more natural non-synthetic ingredients in products. In this review, the main properties of emulsifiers and surfactants will be discussed briefly, followed by an overview of the types of emulsifier and surfactants used currently in consumer product formulations in the pharmaceutical, cosmetic and food industries. Finally, the potential of biosurfactants/bioemulsifiers to replace the currently used counterparts will be assessed.

## **2. Emulsion characteristics and formation**

### ***2.1. Structure and properties of emulsions***

Emulsions are dispersed systems consisting of two immiscible phases (usually water and oil) with one (the dispersed phase) dispersed in the second (the continuous phase) in the form of small droplets [1].

There are two main types of emulsions: water-in-oil (W/O) and oil-in-water (O/W) [2], with notable examples being milk (O/W) or margarine (W/O). Multiple emulsions can also form – W/O/W (water-in-oil-in-water) or O/W/O (oil-in-water-in-oil), with W/O/W found in more applications than O/W/O [3]. These double emulsions are multi-phase systems, where, for example a water-in-oil emulsion is dispersed inside another water phase (W/O/W) where oil droplets that themselves contain dispersed water droplets are dispersed into a second water phase [4]. Double emulsions are of particular interest to the medical, cosmetic and food industries, especially when used for encapsulation of pharmaceuticals (delivery systems for drugs, hormones, steroids), creams (encapsulated active components or vitamins) and

functional foods enriched with nutritional and vitamin-based products and also colours, flavours, minerals and preservatives [5–9].

Emulsions can be classified as macro-, micro- or nano-emulsions, based on the particle size.

Table 1 summarises the main properties of each type of emulsion.

**Table 1.** Properties of different types of emulsions [10].

Parameter	Emulsion	Microemulsion	Nanoemulsion
Formation type	Mechanical or manual agitation	Spontaneous	Ultrasonic and high pressure homogenisation
Stability	Kinetic	Thermodynamic	Kinetic
Droplet size	0.5-50 ( $\mu\text{m}$ )	0.01-0.10 ( $\mu\text{m}$ )	0.05-50 (nm)
Appearance	Cloudy/opaque	Transparent	Transparent or opaque
Surface area ( $\text{m}^2\text{g}^{-1}$ )	Low (15)	High (200)	Moderate (50-100)
Interfacial tension	Low	Ultra-low	Low
System type	W/O, O/W, W/OW, O/W/O	W/O, O/W	W/O, O/W, W/OW
Surfactant concentration	Low	High	Moderate

Special attention has been paid to nano-emulsions for use as emulsion-based delivery systems in foods [11]. One of the main advantages of these systems is the size of the emulsion droplets with the smaller size of the droplets giving higher stability and improved bioavailability [12]. Moreover, nano-emulsions are optically transparent, which makes them suitable for use in juices, water, meat and other foods where a change of colour is undesirable [12,13]. The transparency of nano-emulsions is also beneficial to the cosmetics industry, since the consumer often links products with this character to freshness, simplicity and purity. Nano-emulsions have high stability and products formulated from them do not undergo changes leading to phase separation, which from the consumer's point of view would be a great disadvantage, as it affects the appearance of the product and could negatively influence their future choices. The rheology and delivery (penetration) pathways of nano-emulsions are suited to cream based formulations as they offer increased absorption in different layers of skin, and therefore can be



used to address specific skin issues (such as ceramides in nanoemulsion-based creams for dry skin or atopic dermatitis) [14,15].

## ***2.2. Role of surfactants and emulsifiers in emulsion formation and stability***

Surfactants and emulsifiers play contrasting, but equally important roles in emulsion formation. The main role of surfactants is the lowering of the interfacial tension, which allows formation of small droplets. Emulsifiers, on the other hand, although also surface active, adsorb more slowly to the droplet surface and give longer-term stability to the droplets. Surfactants (and emulsifiers) are able to adsorb at the interface between oil and water, reducing unfavourable interactions between water and oil molecules and allowing dispersion of droplets of one phase in the second [16]. This process leads to a decrease in emulsion droplet size, which results in an increased stability of the emulsion [17,18]. The ability of a surfactant or emulsifier to reduce the surface tension depends on its structure and molecular weight [16]. In addition, the reduction of the interfacial tension (IFT) also depends on the amount adsorbed at the interface with the equilibrium interfacial tension achieved when the surfactant's concentration reaches saturation at the interface [19–21].

One of the factors that determines the selection of surfactants is the relative balance between hydrophilic and hydrophobic regions of the molecule, referred to as hydrophile-lipophile balance (HLB) number [17]. The HLB number is the relative percentage of hydrophilic to lipophilic groups in the surfactant molecule [17]. The HLB number can be used to classify and select low molecular weight (LMW) surfactants and their blends according to their tendency to form o/w or w/o emulsions, with data on HLB of commercial surfactants widely available [22–24]. In general, surfactants with HLB values ranging from 3 to 8 are considered for w/o systems, while o/w emulsions are formed when surfactants of HLB between 9 and 12 are used [25].

There is also a link between HLB number and the aggregation or phase behaviour of the surfactant and therefore its optical appearance (Table 2) [22]. The stability of the emulsions can be directly associated with the HLB values – more stable emulsions can be achieved when the surfactant or its blend has a HLB number similar to that required for the oil phase [26].

**Table 2.** The relationship between HLB, molecular aggregation and appearance in solution [22].

HLB	Aggregation type	Presence in the solution
> 13	Micelles	Clear solution
7-10	Lamellar phase	White dispersion/milky appearance
< 7	Reversed micelles	Lumps of the emulsifier

The HLB determination of biosurfactants is possible once their chemical structure is known. Marqués et al [27] estimated the HLB number of a trehalose tetraester biosurfactant produced by *Rhodococcus* sp. 51T7 to be 11. The authors emphasised the excellent stability of the o/w emulsion when the biosurfactant was used. Mannosylerythritol lipid (MEL) produced by *Candida* sp. SY16 was characterised with HLB of 8.8 by Kim et al. [28] and was suggested by the authors to be used as a potential dispersion agent or o/w emulsifier. Even though some researchers correlate HLB values of synthetic surfactants with biodegradation of hydrophobic compounds (the higher HLB the more efficient biodegradation) [29–31], no such relation was observed for rhamnolipids or synthetic surfactant Triton X-100. These analyses performed by Noordman et al. [32] found HLB of rhamnolipids varying from 17 to 24 (depending on the structure) and as a comparison, HLB 13.4 for Triton X-100.

### 3. The role of emulsions in pharmacy, food and cosmetic industry

#### 3.1. Pharmaceuticals

There is a high demand for emulsions, mostly nanoemulsions, in the pharmaceutical industry. These are formulated as drug delivery systems for the transport of drugs through multiple pathways such as nasal, ocular, oral, parenteral or transdermal [33].

Phospholipids are a good example of emulsifiers used in pharmaceutical technology. Both synthetic and natural phospholipids can serve as components of drugs administered in the form of emulsions, suspensions, complexes or solid dispersions [34]. It is worth mentioning that currently synthetic phospholipids are used only for parenteral/injectable administration, while natural phospholipids can be used for all drug transport routes – including parenteral ones [34].

Table 3 outlines the use of natural and synthetic phospholipids in pharmaceuticals depending on the administration route.

**Table 3.** The use of natural and synthetic phospholipids depending on their administration routes [34].

Phospholipid	Administration Path			
	Inhalation	Oral	Parenteral	Topical
<b>Natural Phospholipids</b>				
<b>Lecithin</b>	+	+	+	+
<b>Soybean lecithin</b>	+	+	–	+
<b>Egg lecithin</b>	–	+	+	–
<b>Hydrogenated soybean lecithin</b>	n/d	n/d	+	n/d
<b>Egg PG</b>	n/d	n/d	+	n/d
<b>Synthetic Phospholipids</b>				
<b>DMPC</b>	–	–	+	–
<b>DOPC</b>	–	–	+	–
<b>DSPC</b>	–	–	+	–
<b>DPPG</b>	–	–	+	–
<b>DSPG</b>	–	–	+	–

Where:

“+” means the administration route can be or is used

“–” the administration route cannot be used

PG – phosphatidylglycerol; DMPC – 1,2-dimyristoylphosphatidylcholine; DOPC – 1,2-dioleoylphosphatidylcholine; DSPC – 1,2-distearoylphosphatidylcholine; DPPG – 1,2-dipalmitoylphosphatidylcholine; DSPG – 1,2-distearoylphosphatidylglycerol; n/d – no data

Emulsion based formulations are of particular use for formulation of drugs with limited water solubility. For example, Choudhury et al., studied the effect of o/w nanoemulsion of paclitaxel – an anti-cancer drug unsuitable for oral delivery as a result of its limited water solubility [35].

The authors used Tween 20 as an emulsifier and a non-ionic and water-insoluble polypropylene

glycol monocaprylate – Capryol 90 as an internal phase to obtain an increased oral bioavailability of paclitaxel nanoemulsion [35].

Bielinska et al. [36] investigated the role of nanoemulsions in the preparation of anthrax protective antigen vaccine based on recombinant *Bacillus anthracis* protective antigen (rPA). The authors used a commercially available soybean oil-water nanoemulsion (NanoBio Corporation, Ann Arbor, MI) and concluded that the nanoemulsion formulations are stable and effective mucosal adjuvants inducing long-lasting, robust and specific cellular responses, with no adverse effects [36].

Nanoemulsions have also been considered and proven successful at the initial development stages of antiretroviral drugs especially for HIV treatment [37–41].

### **3.2. Cosmetics**

The delivery of active compounds in cosmetic industry greatly depends on the emulsion/nanoemulsion formulation. The role of surfactants is crucial in forming these stable systems, also enabling a variety of visual appearances and textures (transparent, opaque, light, or rich/dense) of the products [14]. A careful selection of surfactants for cosmetic formulations is necessary to minimise skin irritations, potential allergic reactions or inflammation as a result of a direct interaction of surfactants with skin keratinocytes [42]. What is more, some surfactants can lead to skin dehydration by depleting intercellular lipids from the stratum corneum [42,43].

Varied surfactants such as non-ionic surfactants – ethoxylated fatty acids and alcohols [44], alkyl polyglucosides [45], polyglycerol esters [46]; amphiphilic oligomers (poly(ethylene glycol) (PEG), oligo(ethylene glycol)) [47,48]; mixtures of fatty alcohols and surfactants [14] or surfactants and polysaccharides [49] can be used in cosmetic formulations.

The use of mixtures or blends of emulsifiers and surfactants is a common practice that can deliver products of the most desired features along with reduced irritation potential. Irritation of the skin is associated with the duration of contact of skin and a surfactant at concentrations below its CMC. The irritation happens because the high concentration of surfactant monomers can lead to the denaturation of the keratin protein in stratum corneum (the outermost skin layer) [50]. Therefore, it is generally favoured to use surfactant mixtures that are characterised by lower CMC and hence limited irritation potential [50]. The components of the surfactant/emulsifier mixtures can be added separately during the emulsification process or in the form of a pre-manufactured blends (also called – emulsifying waxes) [51–53]. A good example of such blends is a phosphate-based self-emulsifying wax consisting of cetaryl alcohol, dicetyl phosphate and ceteth-10 phosphate or non-ionic self-emulsifying base (with cetaryl alcohol and cetareth-20) commonly used in sunscreens [54,55]. Table 4 lists the most common surface-active agents used in cosmetics, along with their functional properties.

**Table 4.** Surface-active agents commonly used in cosmetic formulations [56–60].

<b>IUPAC notation</b>	<b>INCI* notation</b>	<b>Function</b>
12-hydroxyoctadecanoic acid	12-Hydroxy Stearic Acid	Emulsifier, emulsion stabiliser, surfactant, viscosity regulator
Docosan-1-ol	Behenyl Alcohol	Emollient, emulsifier, thickener
Hexadecan-1-ol; octadecan-1-ol	Ceto Stearyl Alcohol	Emulsifier, emulsion stabiliser, viscosity regulator, foam enhancer
Hexadecan-1-ol	Cetyl Alcohol	Emulsifier, emulsion stabiliser, viscosity regulator, foam enhancer
Hexadecyl hexadecanoate	Cetyl Palmitate	Emulsifier, emulsion stabiliser, viscosity regulator
Propan-1,2,3-triol	Glycerine	Humectant
2,3-dihydroxypropyl octadecanoate	Glyceryl Monostearate	Emulsifier, emulsion stabiliser
2-octadecanoyloxyethyl octadecanoate	Glycol Distearate	Emulsifier, emulsion stabiliser

2-hydroxyethyl octadecanoate	Glycol Stearate	Emulsifier, emulsion stabiliser
2,3-bis(12-hydroxyoctadecanoyloxy)propyl 12-hydroxyoctadecanoate	Hydrogenated Castor Oil	Emulsifier, emulsion stabiliser, viscosity regulator
Propan-2-yl tetradecanoate	Isopropyl Myristate	Emollient, thickening agent
Dodecanoic acid	Lauric Acid	Emulsifier, emulsion stabiliser
[(2~{R})-3-hexadecanoyloxy-2-[(9~{Z},12~{Z})-octadeca-9,12-dienoyl]oxypropyl] 2-(trimethylazaniumyl)ethyl phosphate (for Soybean lecithin)	Lecithin	Emulsifier, emulsion stabiliser, viscosity regulator
Macadamia oil	Macadamia Ternifolia Seed Oil	Emollient, emulsifier
Tetradecanoic acid	Myristic Acid	Emulsifier, emulsion stabiliser
(Z)-octadec-9-enoic acid	Oleic Acid	Emulsifier, emulsion stabiliser
Hexadecanoic acid	Palmitic Acid	Emulsifier, emulsion stabiliser, viscosity regulator
– (Coconut Glycerides, Ethoxylated)	PEG 7 Glyceryl Cocoate	Emulsifier, surfactant, emulsion stabiliser, foam enhancer
Sodium;2-(dodecanoylamino)acetate	Sodium Cocoyl Glycinate	Emulsifier, emulsion stabiliser, surfactant, foam enhancer
Sodium;2-butanoyloxyethanesulfonate	Sodium Cocoyl Isethionate	Surfactant, foam enhancer
[2-(3,4-dihydroxyoxolan-2-yl)-2-hydroxyethyl] octadecanoate	Sorbitan Stearate	Emulsifier, emulsion stabiliser, surfactant,
2-octadecoxoethanol	Steareth-2	Emulsifier
Octadecanoic acid	Stearic Acid	Emulsifier, emulsion stabiliser, viscosity regulator
Octadecan-1-ol	Stearyl Alcohol	Emulsifier, emulsion stabiliser, viscosity regulator, foam enhancer

\*INCI – International Nomenclature of Cosmetic Ingredients

### 3.3. Food

The vast majority of food emulsions represent very complicated systems with a variety of ingredients such as acids and bases, alcohols, carbohydrates, free fatty acids, flavours, mono-;

di- and tri-acylglycerols, preservatives, proteins, salts, surfactants, vitamins etc. The complexity of the food emulsions not only depends on their components but also on the production and storage conditions (changes in temperature, pressure, pH, oxygen limitations etc.) which can lead to important changes in the properties of the overall systems [1,61]. Two other important factors considered in the production of food emulsions are their flavour and visual appearance. Both features strongly depend on the droplet size distribution of the emulsion systems. Smaller droplets not only lead to thermodynamically more stable emulsions, but also ones less prone to bacterial growth. This is because smaller droplets limit nutrient availability in droplets for bacterial growth and reproduction [62,63].

The composition (e.g. lipid content), structure (droplet size) and stability of emulsions are the main factors that can have a significant impact on the digestion behaviour, which is relevant to their nutritional properties. In recent years the relation between emulsion physicochemical properties and fat digestion (lipid uptake and metabolism) has gained significant attention [64–67]. Zhang et al. [68] showed differences in the digestibility of oil-in-water emulsions containing various oils as the dispersed phase– vegetable (corn, olive, sunflower, canola), marine (fish and krill), flavour oils (lemon and orange) and medium chain triglycerides. The authors concluded that the digestibility of the emulsions was related to structural differences in the oils – unsaturation or fatty acid chain length, and emulsion features such as particle size, charge and appearance which depend on the oil type [68].

An application of growing technical importance is the design of emulsions with special carrier features, such as delivery of vitamins, antimicrobials, antioxidants and microelements in stable, efficient and easily accessible forms. McClements et al. [7] emphasise the importance of successful delivery of nutraceuticals of proven health benefits. These include carotenoids (lutein and zeaxanthin for the prevention of cataracts and other age-related eye degradation; lycopene, which is believed to decrease the risk of prostate cancer), conjugated linoleic acid

(CLA), omega-3 ( $\omega$ -3) fatty acids, proteins, amino acids, phytosterols, or minerals such as zinc, iron, calcium, selenium or chromium.

The role of the emulsifiers in food emulsions is to adsorb to the droplet surface during the production and to prevent droplet coalescence and aggregation. They do this by forming a steric stabilizing adsorbed layer at the droplet surface. In particular, protein-polysaccharide mixtures have been studied with regards to emulsion stabilisation. Structures containing mixes of whey protein isolate (WPI) and a bioemulsifier xanthan gum, as well as whey protein concentrate and pectin proved to be most efficient stabilisers [4].

Surfactants (usually low molecular weight components) should improve the emulsion formation but can also alter its initial features such as looks, growth, enhance or prevent the interactions with other emulsion components etc. [1,65].

**Table 5.** Most common food emulsifiers and surfactants [1,69,70].

Chemical name	Abbreviation
Lecithin	—
Fatty acid salts	FA
Sodium stearyl lactylate	SSL
Calcium stearyl lactylate	CSL
Citric acid esters of MG	CITREM
Diacetyl tartaric acid esters of MG	DATEM
Monoglycerides	MG
Acetic Acid esters of MG	ACETEM
Lactic acid esters of MG	LACTEM
Succinic acid esters of MG	SMG
Polyglycerol esters of FA	PGE
Propylene glycol esters of FA	PGMS
Sucrose esters of FA	—
Sorbitan monostearate	SMS
Sorbitan tristearate	STS
Polyoxyethylene (20) sorbitan monostearate	Polysorbate 60
Polyoxyethylene (20) sorbitan tristearate	Polysorbate 65



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Polyoxyethylene (20)  
sorbitan monooleate

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Polysorbate 80

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Mono- and diglycerides play an important role in the food industry (Table 5). They are produced by interestrification (glycerolysis) of edible fats or oils with glycerol under alkaline pH and high temperatures 200-260°C [70,71]. Monoglycerides are oil-soluble, polymorphic and can therefore exist in various crystalline forms depending temperature and concentration [71,72]. They can be found in bread, cakes, sponge cakes, desserts, margarines, spreads, ice creams, chewing gum etc., although approximately 60% of all monoglycerides are used in bakery [71]. Another group of important surfactants in food industry constitute polyol esters of fatty acids. The most common polyols used for esterification in this case are polyglycerol, propylene glycol, sorbitan, polyoxyethylene sorbitan, and sucrose, while the selection of fatty acids varies depending on their degree of unsaturation and the chain length (12-18 carbon atoms mostly) [1,69,70].

#### **4. Bioemulsifiers and biosurfactants**

Bioemulsifiers and biosurfactants – surface-active agents of microbial origin have been recently named as the multifunctional biomolecules of the 21<sup>st</sup> century [73]. They are not only a sustainable alternative for chemically synthesised surfactants, but also offer enhanced performance under extreme conditions (temperature, pH, salinity etc.) [74]. Furthermore, low toxicity, biodegradability and environmental compatibility of biosurfactants are additional advantages over their chemical counterparts. Due to their varied functionality (emulsion forming and stabilising, foaming, lowering of surface and/or interfacial tension) as well as in some cases antiviral, antifungal and antibacterial activities, biosurfactants find applications in many branches of industry. Applications range from bioremediation of organic pollutants in soil and water, through cleaning and washing agents, food and cosmetics, and pharmaceutical and bio-medical [75–80]. Biosurfactants with the highest potential for exploitation in these applications are the glycolipids and lipopeptides. For bioemulsifiers, heteropolysaccharides,

lipopolysaccharides, lipoproteins, glycoproteins and proteins are considered valuable for industrial applications. In the last three years, a number of new applications of the new and already known biosurfactants/bioemulsifiers have been reported. Table 6 summarises some of the most interesting findings.

**Table 6.** The latest (2016-2019) findings on biosurfactants and bioemulsifiers of microbial origin

<b>Biosurfactant/ bioemulsifier</b>	<b>Strain</b>	<b>Activity</b>	<b>Potential Industrial Application</b>	<b>Reference</b>
MELs	<i>Pseudozyma</i> sp.	Anti-melanogenic effect.	Cosmetics. Anti- melanogenic agent for effective skin whitening	[81]
Sophorolipids	(obtained from a commercial source)	inhibition/disrupti on of biofilms formed by Gram- positive and Gram-negative microorganisms	Pharmaceuticals, Food preservation, Food packaging	[82]
Iturin A	<i>Bacillus</i> <i>megaterium</i>	Anti-cancer (human breast cancer)	Medicine	[83,84]
Surfactin	<i>Bacillus</i> <i>subtilis</i>	Anti-viral, Membrane fusion inhibitor,	Medicine	[85]
Surfactin	<i>Bacillus</i> <i>amylolique- faciens</i> ST34	Anti-bacterial and anti-fungal activity	Medicine and food as antimicrobial agents	[86]
Mono/di- rhamnolipids	<i>Pseudomonas</i> <i>aeruginosa</i> ST5	Anti-bacterial and anti-fungal activity	Medicine and food as antimicrobial agents	[86]
Lipopeptide	<i>Bacillus</i> <i>subtilis</i> SPB1	Anti-oxidant, wound healing properties	Medicine, Pharmaceuticals	[87]
*EPS	<i>Bacillus</i> <i>vallismortis</i> WF4	Emulsifier, Emulsion stabiliser, In the emulsion with essential oils it had antifungal and antipruritic properties	Medicine, Pharmaceuticals, Food industry	[88]

The exopoly-saccharide EPS273	<i>Pseudomonas stutzeri</i> 273	Anti-biofilm (especially against <i>P. aeruginosa</i> ), Anti-fouling	Agent against bacterial biofilm-associated infections, Food-processing contamination, Marine biofouling	[89]
EPS	<i>Rhodobacter johrii</i> CDR-SL 7Cii	Emulsifier, Emulsion stabiliser	Food industry, Cosmetics	[90]
EPS	<i>Pseudomonas fluorescens</i>	Emulsifier	Cosmetic formulations, Bioremediation	[91]
EPS	<i>Chromohalobacter canadensis</i> 28	Emulsifier, Emulsion stabiliser, Foamer	Cosmetics, Food industry	[92]
Lipopeptide	<i>Nesterenkonia sp.</i> MSA31	Anti-oxidant, Anti-biofilm, Emulsifier, Emulsion stabiliser	Food industry	[93]
Glycolipo-protein	<i>Acinetobacter indicus</i> M6	Anti-proliferative activity against lung cancer, Anti-biofilm, Anti-bacterial	Pharmaceuticals, Medicine, Food control	[94]
Glycolipo-peptide	<i>Lactobacillus pentosus</i> (PEB)	Anti-microbial Anti-adhesive	Cosmetic and personal care formulations, Medicine	[95]
Glycolipo-peptide	<i>Lactobacillus paracasei</i>	Stabilizing agent in oil-in-water (cosmetic) emulsions	Cosmetics, Food	[96]
**LAB-biosurfactant	<i>Lactobacillus helveticus</i> MRTL91	Anti-microbial, Anti-adhesive against pathogens	Biomedical, Pharmaceuticals, Cosmetics, Food	[97]
LAB-biosurfactant	<i>Lactobacillus casei</i>	Anti-oxidant, Anti-proliferative activity on epithelial cells, Anti-adhesive, Anti-biofilm	Pharmaceuticals, Cosmetics, Medicine, Food and food packaging	[98]

\*EPS – exopolysaccharides

\*\*LAB-biosurfactant – lactic acid bacteria (LAB)-derived biosurfactant

An important factor for commercialising biosurfactants or bioemulsifiers is whether they are secreted outside the cell or if they are cell-bound. In case of the latter, the purification process needs to be enhanced with additional steps for successful extraction of the bioactive molecules [99]. Glycopeptides (or glycolipopeptides) are a good example of cell-bound biosurfactants and are gaining more attention due to their anti-microbial and anti-adhesive properties. They are produced by LAB such as *Lactobacillus pentosus* [95,100].

On the other hand, EPS are well known extracellular surface-active molecules of varied chemical structures and functionality [101]. Their role in biofilm formation has been extensively studied together with their potential in bioremediation strategies [102–105]. However, lately EPS has gained additional interest with respect to their application in dairy products as natural stabilisers and thickeners [106,107]. Other example applications of EPS are listed in Table 6.

#### **4.1. Biosurfactants - glycolipids**

Glycolipids are an interesting and diverse group of biosurfactants. They contain a sugar-based hydrophilic part attached through a glycosidic bond to a hydrophobic moiety, usually a hydroxy fatty acid. The most studied representatives of this class of biosurfactants are rhamnolipids and sophorolipids [108,109]

##### **4.1.1. Rhamnolipids**

Rhamnolipids (RLs) are undoubtedly the best-known biosurfactants. RLs were originally extracted from an opportunistic pathogen – a gram-negative bacterium *Pseudomonas aeruginosa*, and this is still a major source of the biosurfactants. The pathogenic nature of the principal producing organism has held back the application of RLs in foods and cosmetics, and effort has been put into finding non-pathogenic sources.

RLs have been extensively studied over the past 30 years for their role in biodegradation of hydrocarbons, including polycyclic aromatic hydrocarbons (PAH) [110–115]. Apart from their ability to enhance a dispersion of the organic pollutants, RLs were also found to affect the cell surface hydrophobicity of the oil-degrading bacteria, which in some cases positively affected the biodegradation rates, and in others completely inhibited it [110,116–118].

Following the ability of RLs to modify surface properties of microorganisms, their antimicrobial and anti-biofilm formation were studied by various researches. Elshikh et al. [119] investigated the antimicrobial properties of RLs extracted from the non-pathogenic bacteria *Burkholderia thailandensis* E264 against selected oral pathogens such as *Streptococcus oralis*, *Actinomyces naeslundii*, *Neisseria mucosa* and *Streptococcus sanguinis*. The significant reported reduction in bacterial viability of these strains, placed RLs as a prominent additive for oral care formulations and devices [119]. Magalhães and Nitschke [120] evaluated the antimicrobial potential of RLs against *Listeria monocytogenes* - a foodborne pathogen. This Gram-positive bacteria can be found in various types of food ranging from raw meat and vegetables to ready meals and other refrigerated foods, as well as on floors, drains and equipment used for food production and processing [120–123]. Rhamnolipids extracted from *Pseudomonas aeruginosa* strain showed an antimicrobial effect against *Listeria monocytogenes*, which was enhanced by the presence of the antimicrobial peptide nisin [120].

Antimicrobial activity of RLs against the most common food pathogens (*Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enteritidis*) at different pH values has recently been studied by de Freitas Ferreira [124]. Based on the high activity of RLs against *B. cereus*, *L. monocytogenes* and *S. aureus*, the authors emphasised the potential of RLs in strategies for control of pathogens in food processing.

Antimicrobial activity of rhamnolipids is not only beneficial in the food processing industry, but has also been considered as a valuable feature in biopesticides production. The use of rhamnolipids in biopesticide composition was described in several patent applications [125,126].

According to DeSanto, RLs mixed with fertilizers can be injected or sprayed into the stem, root, trunk, or branch of a plant, a bush or a tree to cure disease [127]. For that purpose, the American company Rhamnolipid Inc. offers RL-based products, mainly to cure cannabis plant diseases [128].

As a food additive, RLs were a subject of a patent application by Van Haesendonck et al. [129], as an improver of dough or batter stability, volume, shape and texture of dough, width of cut and/or microbial conservation of bakery products. According to the application, the RLs can be further used to improve the properties of butter cream, decoration cream and/or of non-dairy cream filling for Danish pastries, croissants and other fresh or frozen fine confectionery products [129].

Most commercial applications of RLs are for household cleaning purposes and remediation strategies. Logos Technologies LLC (USA) launched a RL-based product called NatSurFact<sup>®</sup>, which, according to the company, can be applied in many branches of industry such as personal care, industrial and household cleaning, medicine, agriculture, wastewater treatment, oil recovery and bioremediation [130]. Another American company, AGAE Technologies provides 90% and 95% pure rhamnolipids for potential use in pharmaceuticals, cosmetics, oil tank cleaning, laboratory reference standards, personal care products, home cleaning products, enhanced oil recovery and environmental remediation [131].

High purity RLs are beneficial in cosmetic-based formulations especially for wrinkle treatment [132]. According to the author, RL mixed with hyaluronic acid results in much higher concentration of collagen in the skin compared to HA used alone.

For biomedical applications, the excellent surfactant properties of rhamnolipids give the possibility to use them for increasing the bioavailability of hydrophobic antibiotics, but since they possess antimicrobial activity themselves, they have been described as possible agents in treating drug-resistant bacterial pathogenic infections [133,134].

The discovery that RLs readily absorb into the blood stream when topically applied onto the skin, has helped to understand the mechanism of RL's positive effect on radiation burn treatments, as stated in the patent application by DeSanto [135].

Wound healing properties of RL compositions along with effect on gum disease and periodontal regeneration were also the subject of a 2010 patent by Stipcevic et al. [136]. Rhamnolipids were discovered to have a beneficial influence on burn wound healing, accelerating the wound closure as well as reducing the healing failures in chronic wounds [136,137].

#### **4.1.2. Sophorolipids**

Sophorolipids (SLs), first described in 1961 by Gorin et al [138], are glycolipid biosurfactants composed of a sophorose (hydrophilic moiety) and a hydroxylated fatty acid ( $C_{16}$  or  $C_{18}$ ) as the hydrophobic part. There are two main forms of sophorolipid structures – lactonic and acidic with differing degrees of acetylation, leading to different functional properties. They are produced by a number of non-pathogenic yeast species such as *Starmerella*, *Candida* and *Pseudohyphozyma*, which is an important advantage for the application of these surfactants in food- or medical-related products [139]. A post-fermentation product usually contains a

mixture of SLs, reaching nearly 20 molecules, however only a few congeners are produced in significant amounts [140].

Sophorolipids are able to form emulsions with a range of hydrocarbon and triglyceride oils, suggesting their potential use in the food, cosmetics and petroleum industries. Sophorolipids (mixed lactonic, acid, and esterified forms) have been compared to Triton-X, a non-ionic surfactant, for their ability to emulsify Arabian light crude oil, and various pure hydrocarbon oils [141]. Not only were the SLs able to form oil-in-water emulsions, but also under some conditions they formed water-in-oil emulsions. The SLs, and in particular the esters, outperformed Triton-X in all tests. Additionally, the SL esters displayed the ability to reduce the size of a crude oil slick on water by 20% of its original area, a property useful if the slick is to be burned off. Differing chain lengths of the alkyl group enable tuning of the HLB of the SLs properties, with longer chain lengths giving lower HLB numbers as the hydrophobic character increases. The same research group has investigated the use of modified alkyl esters of sophorolipids as stabilisers of paraffin oil, almond oil and lemon oil emulsions [142–144].

A Germany-based company Evonik is the first European company to have produced and commercialised sophorolipids on an industrial scale [145]. Their first sophorolipid product called REWOFORM<sup>®</sup> SL is already commercially available for the use in detergents and home care cleaning products [146]. In 2016 Evonik received an EPAWA Innovation Award 2016 for the “the outstanding cleaning properties and the excellent ecological profile of the Rewoform biosurfactant” [147]. According to Evonik, REWOFORM<sup>®</sup> SL fills the requirements of OECD 301F and EN ISO 11734 for rapid biodegradability under both aerobic and anaerobic conditions, has an outstanding Renewable Carbon Index (RCI) of 100 percent and performs significantly better than other surfactants when tested for the water toxicity (OECD 211 and 202) [146]. Sophorolipid production using genetically modified yeast cells in order to increase the yields and reduce the production costs by Evonik and was successfully granted a US Patent



in 2015 [148]. An extensive study on the use of sophorolipids in other possible branches of industry has led Evonik to file a patent application entitled “Increasing the yields by the use of sophorolipids” [149]. The invention describes the advantageous strategies of using sophorolipids for increasing the yields of agriculturally useful crop plants (e.g. wheat, maize, oats, rye, barley, soybeans, garden beans, potatoes, sunflower, oilseed rape, tomatoes, lettuce, cabbage, fruit trees, plantation crops like coffee and tea). According to the invention, one of the main advantages of using of sophorolipids is the reduction or total elimination of the use of pesticides and fungicides. Sophorolipids can be used as adjuvants in pesticide formulations, limiting their concentrations and therefore the overall costs.

Ecover, a Belgian manufacturer of cleansing and detergent products introduced sophorolipids from Evonik in their multi-purpose cleaning products [150], which are available in shops and online with excellent reviews from the customers [150,151].

#### ***4.1.3. Other glycolipids***

Other known representatives of glycolipids family are the threhalolipids (TLs, also known as trehalose lipids) and mannosylerythritol lipids (MELs).

Despite their excellent physicochemical properties and surface activity, the use of TLs in industry is limited due to the scale-up difficulties. This is a result of their dependence on particular carbon sources (which in most cases is long chain aliphatic hydrocarbon such as hexadecane), low yields, and high costs of recovery of the surfactant from the fermentation broth due to its association with the bacterial cell wall [152,153].

Nonetheless, most bacterial strains producing TLs have been isolated from oil-polluted sites and showed very good efficiency in biodegradation of hydrocarbon pollutants. Therefore, the main applications of TLs to date is in the petroleum industry, microbial enhanced oil recovery, cleaning of oil storage tanks and hydrocarbon bioremediation [109]. Other properties of TLs

suggest their possible use in biomedical research. They interact with cell membranes, inhibit the growth of the human leukemic cell lines and have some antiviral and antifungal properties [154]. Isoda et al. [155] reported that TLs inhibit protein kinase C (PKC) activity in the human promyelocytic leukemia cell line HL60, which is an important feature of anti-tumour agents. The authors also observed the role of TLs in inducing the cell differentiation of the HL60 to monocytes.

The general structure of MELs comprises a 4-O- $\beta$ -d-mannopyranosyl meso-erythritol as the hydrophilic moiety attached to a hydrophobic fatty acid chain [156]. Depending on the acetylation degree at the hydrophilic group, four main classes of MELs are known – the diacetylated MEL-A, monoacetylated MEL-B and MEL-C or non-acetylated MEL-D [156].

MELs are produced by yeasts of *Pseudozyma*, *Ustilago* and *Schizonella* species [157]. The moisturising effect of MEL-A on dry and damaged skin was first reported by Morita et al. [158], who compared the moisturising properties of the biosurfactant to the natural ceramides. The latter due to their excellent hydrating properties have been applied in numerous skin-care formulations, however, the costs of commercial use of the natural ceramides is high as a result of their limited availability and sophisticated extraction-purification methods [159]. Therefore, the discovery of the ceramide-like properties of MELs has led to patented application of the biosurfactant in skin-care products [160,161]. Currently, MELs for cosmetic applications are commercially available as SurfMellow<sup>®</sup> produced by the Japanese Company - Toyobo Co., Ltd. [162], which is also available in the United States [163].

#### **4.2. Biosurfactants – lipopeptides**

Lipopeptides (LPs) belong to the class of surface-active molecules constituting a fatty acid chain (of varied lengths) and peptide moiety. Depending on their structures, lipopeptides can be divided into several isoforms, such as surfactin, iturin, fengycin, plipastatin, bacillomycin,

viscosin, tensin, arthrofactin, pseudofactin and syringomycin, to name a few and it is not unusual for a single microorganism to produce more than one isoform [164]. Lipopeptides can be produced by various types of aerobic microorganisms – bacteria, yeasts, molds and actinomycetes [164]. One of the best known lipopeptides is surfactin produced by the gram-positive bacterium *Bacillus subtilis*. Surfactin is of interest due to its anti-bacterial, anti-viral, anti-fungal and anti-mycoplasmal activities. It is also known to inhibit fibrin clotting and as a cell lysate promoter [165]. Surfactin was also discovered to have anti-aging, anti-photoaging, anti-wrinkle and anti-oxidation repairing functions along with enhanced skin penetration activity and can also induce the production of collagen in the skin. All of these findings were described in the patent applications by Lu et al. [166,167]. The same authors patented the formulations for anti-aging formulations, which are currently being explored by a Taiwanese company UMO International [168,169].

Currently surfactin (INCI name: sodium surfactin) is available through the Japanese dermatology company KANEKA [170] for cosmetic formulations. Kaneka together with the Italian company Sabo released their first surfactin-based product in 2015, a lipid layer enhancer SABO®SOL RF [171].

The other well-known LPs produced by *Bacillus* species are iturin and fengycin, whereas *Pseudomonas* species are known to produce tensin, pseudofactin, viscosin and polymyxin (an antibiotic used for various gram-negative bacterial infections). Two significant LP antibiotics – amphotericin and laspartomycin are produced by *Streptomyces* species. Amphotericin is a powerful antibiotic used against invasive fungal infections in humans – i.e. aspergillosis and candidiasis and also *Leishmania* parasites [172,173]. Laspartomycin has activity against vancomycin-resistant enterococci and methicillin-resistant *Staphylococcus aureus* [174,175].

Thanks to their excellent antimicrobial and antiviral properties, LPs play important roles in pharmaceuticals, cosmetics and agriculture. Surfactin has been reported as an effective agent against a wide range of phytopathogens, including those that have developed a resistance to other specific antimicrobial agents [176]. Lipopeptides in cosmetics serve as active ingredients in anti-wrinkle, moisturising and cleansing formulations, and they have been shown to interact with lipopolysaccharides, leading to the suppression of lipid transportation, which suggests potential for cellulite treatment [177]. The LPs also have potential benefits to the food industry as efficient emulsifiers, texture modifiers and stabilisers [178,179].

### **4.3. Bioemulsifiers**

#### **4.3.1. Emulsan**

Emulsan is an extracellular heteropolysaccharide, complex of two biopolymers – 80% lipopolysaccharide and 20% high molecular weight exopolysaccharide [180]. Extracted from a hydrocarbon-degrading *Arthrobacter* sp. RAG-1 (later identified as *Acinetobacter venetianus* RAG-1) in the late 70s [181,182], emulsan was identified for its exceptional emulsion forming and stabilising properties especially in relation to hydrocarbon biodegradation [183,184]. For the next three decades, emulsan was valued as an important biopolymer in bioremediation techniques [185]. However, it seems that the attention has now turned to its possible use in pharmacy and medicine. The work presented by Panilaitis et al. [186] highlighted the adjuvant activity of emulsan, especially its activity to activate macrophages, which was dependent on the structural variants of emulsan. The significant immunopotentiality obtained for emulsan formulations makes this biopolymer an important candidate for new types of adjuvants.

The natural ability of emulsan to bind proteins has been investigated in mixed biopolymer controlled molecule-release systems. Emulsan addition proved to have a positive effect on the stability of microspheres of alginate, giving the possibility of tuning the release of biological

molecules by applying different concentrations of emulsan. Based on their findings, the authors suggested emulsan as an excellent candidate for delivery of proteins and pharmaceuticals [187,188]. Specific emulsan-alginate compositions were patented as drug-delivery systems and vehicles for removing protein-based toxins from food products and/or other products [189].

The recent study carried out by Yi et al. [190] has investigated the nanoparticles formation using emulsan as a coating for hydrophobic oil core into which a model drug Pheophorbide (Pba, a photosensitizer) was loaded. These nanoparticles presented a fast uptake in mouse carcinoma cells and successful tumour kill after laser radiation due to the photodynamic effect of Pba. What is more, the accumulation of the model nanoparticles in tumour tissues was several times higher when compared to the free Pba accumulation, showcasing the potential of emulsan to be used in formulations of nanoparticles for anti-tumour drug-delivery systems [190].

Emulsan production has not yet been commercialised and therefore this bioemulsifier is not available for customers. However, a different bioemulsifier, of plant origin - Methyl Glucose Sesquistearate (INCI nomenclature) is available under the name of Emulsan and is used mostly for cosmetic purposes [191]. Despite sharing the same name, the two emulsifiers are completely different in terms of their chemical composition and origin and should not be confused.

#### **4.3.2. Alasan**

Alasan is a complex of anionic polysaccharides containing covalently bound alanine-rich proteins, produced by *Acinetobacter radioresistens* [192]. The three major proteins of alasan have molecular masses of 16, 31, and 45 kDa and have been associated with the emulsifying and surface activity of alasan [193]. According to Toren et al. [194] the protein of 45 kDa showed the highest emulsifying activity, which was in fact higher than the intact alasan

complex. What is more, the authors obtained further increase in emulsifying activity and stability of oil-in-water emulsion when the purified 16 and 31 kDa proteins were added to 45 kDa protein. Despite its emulsifying potential, alasin has yet to find wide application other than bioremediation strategies, especially in the field of PAH mineralisation [108,195–197].

#### **4.3.3. Xanthan Gum**

Xanthan gum (XG), produced by bacteria of the *Xanthomonas* genus, is the most widely used commercial polysaccharide of microbial origin [198]. The role of xanthan in many branches of industry is continuously growing and according to Grand View Research Inc., the global xanthan gum market is expected to reach USD 987.7 million by 2020 [199].

Structurally, XG is a heteropolysaccharide consisting of glucose, mannose and glucuronic acid in a 2:2:1 ratio with pyruvate and acetyl substituent groups [200]. The main chain (backbone) of XG is formed of  $\beta$ -D-glucose linked at position 1 and 4, which makes it nearly identical to cellulose [200]. Despite this similarity, XG is resistant to cellulase enzymes [201]. These features make xanthan gum a stable thickening and stabilizing agent even in cellulase-rich systems. Since it is not digestible by humans, XG can be used to lower the calorific values of foods, as its own calorific value is about 0.6 kcal/g [201].

Xanthan gum is non-toxic and non-sensitizing, as it does not cause eye or skin irritation. Thus it was approved by the United States Food and Drug Administration (FDA) for use as a food additive without any specific quantity limitations [202]. In 1980, it was added to the food emulsifiers/stabilizers list as E-415 by the European Economic Community [202].

As a thickening agent, XG is used in soups, salad dressings, gravies, sauces, toppings etc., what is more, due to its ability to form gels as a result of synergistic interactions with polysaccharides (especially of galactomannan family), xanthan gum is used also as a gelling and viscosity modifying agent [203]. The stability of XG over a broad range of temperatures, salt contents

(up to 150 g/L NaCl) and pH (2-11) makes it a valuable component in biomedical and cosmetic applications [204]. Kumar et al. [205] report XG as a biomaterial for tissue scaffolds in tissue engineering applications. A formation of phospholipid (1,2-dioleoyl-sn-glycero-phosphoethylamine, DOPE) – xanthan self-assembling conjugate further fabricated to microcapsules was reported by Mendes et al [206]. The authors showed the microcapsules provided a good environment for cellular survival and proliferation, which is a promising feature for cell-based transplantation therapy applications [206]. Pooja et al. [207] explored XG for gold nanoparticles synthesis. The authors reported the obtained nanoparticles to be stable at pH 5-9 and NaCl concentration up to 0.5M and when tested in serum, a significant stability up to 24h was noted. Furthermore, toxicity studies proved the XG-Au nanoparticles to be biocompatible and non-toxic, which together with their stability features make the presented synthesis of gold nanoparticles a better alternative to traditionally synthesized gold nanoparticles (GNP) with harsh and toxic chemicals [207].

## **5. Conclusions and future perspectives**

Biosurfactants and bioemulsifiers are perfect alternatives to their chemical counterparts due to their low toxicity, exceptional physicochemical characteristics (incl. surface/interfacial activity), bioavailability and renewable-resource origin. These features make the bio-based surface active agents highly interesting for the main industries linked to human health – pharmaceuticals, food and cosmetics, as well as environmental protection and as stated in this review, many have already been widely applied. However, the commercialisation of the production of bio-surfactants/emulsifiers is often limited by the optimisation difficulties, purification costs, low yields and variations in product features, all of which has an impact on their higher prices when compared to the chemical surfactants. Therefore, a lot of effort is required to find stable and cost effective methods to obtain biosurfactants and bioemulsifiers on a large scale. Genetic engineering can help with regards to increasing the production yields

and reproducibility of the surface active compounds, however, products from genetically modified organisms (GMO) are not favoured by consumers, which greatly limits their applications in cosmetic/personal care and food industries. Nonetheless, biosurfactants and bioemulsifiers have an undeniable potential for replacing the conventional surfactants with great benefit to human health and environment and given the fast-development in chemical/biotechnological engineering, it is just a matter of time.

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